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# Establishing Microbial Contamination and Biocides Efficacy Evaluation in Cooling Tower Water Samples

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## ABSTRACT

Cooling towers system is a critical unit in many industrial production processes, including oil industry, as they provide the most efficient means of rejecting heat from open re-circulating cooling systems. The basic objectives of a cooling water treatment program are to increase process efficiency, production output & maximize equipment service life.

Recirculating cooling water is susceptible to microbial contamination through incoming water and air. An inappropriate control of these microorganisms may lead to biological fouling in heat exchangers, which enhance scale formation and corrosion, impacting heat transfer efficiency and treatment, which affects overall process efficiency and result in the adverse effects on production.

The paper documents the results of investigations with respect to the degree of microbial invasion in the cooling water systems of LPG-I,II, C2-C3,APU units of Uran Plant. It also includes evaluation of biocides efficacy for effective control of the microbial proliferation against the presently used doses.

The study has revealed that the presently used doses of biocides were not effective to control heavy load of microbes present in the system. Therefore, efficient doses of biocides were evaluated through Minimum Inhibitory Concentration method. All the biocides were effective in the range of 50-100 ppm for LPG-I, II and C2-C3 whereas for APU it was above 150 ppm. It has been recommended to implement these doses for complete microbial control in the system.

*Keywords: Sulphate-reducing bacteria (SRB), General aerobic bacteria (GAB), TVC-Total Viable Count, MIC- Minimum Inhibitory Concentration, Quat (Quaternary ammonium type), MBT (Methylene Bis Thiocyanate), DBNPA (2,2 Dibromo-3 nitrilo propionamide).*

## INTRODUCTION

Cooling towers are an integral part of large range of industrial production processes[1]. Open recirculating cooling water systems provide an ideal environment for microbial growth and biofilm formation. Micro-organisms present in cooling towers can be separated into two distinct but related populations. Firstly, the microbial flora in the planktonic phase which may be transient or actively multiplying, and secondly, the microbial flora in biofilm. Microorganisms which enter via makeup water or air to the system form a biofilm layer on the inside surfaces of cooling tower. Biofilms are the product of adhesion and growth of microorganisms on surfaces. On one hand, biofilm layer acts as a biological filters providing mechanical stability and also protects its inhabitants from physico-chemical alterations occurring in the bulk water phase. On the other hand, biofilms in cooling towers may lead to many undesired conditions such as equipment damage through corrosion, decreased energy efficiency due to increased hydraulic pressure (pumping costs), local blocking of cooling towers and increased heat transfer resistance[2]. Apart from biofilm formation, fouling is yet another major problem associated with the cooling towers leading to problems similar to that of biofilm formation. Fouling is the accumulation of deposits on surfaces in contact with an aqueous solution and different types of fouling are found in cooling water systems, including biofouling which are caused by organisms (micro- and macro-organisms) found in the system.

One of the major concerns of process cooling water systems is microbiologically induced corrosion[3] (MIC). MIC in water systems is due to by-products of microbes that are corrosive. They are acids, alkalis or reducing agents such as $H_2S$, ammonia, sulfuric acid and organic acids. These by-products or wastes lead to under deposit corrosion.

The control of microbial flora in circulating water systems has taken an added importance since the discovery of hygienically relevant pathogenic bacteria *Legionnella*, responsible for 'Legionellosis'[4] which can be spread into the environment through aerosols generated in the cooling tower.

Because of the economic loss, operational and public health reasons, both bulk water and biofilm layer of cooling towers should be monitored and controlled in view of microbial burden. Control measures involve proper monitoring for the detection of specific microorganisms responsible for the damages and use of chemicals to control them[5].

Cooling Towers of LPG-I, LPG-II, C2-C3 and APU units of Uran Plant, Oil and Natural Gas Corporation (ONGC), maintain the heat efficiency through the circulation of cooling water with the help of heat exchangers. In order to maintain the overall efficiency and to control the microbial growth in the system biocides are employed in all the cooling tower systems. Regional Geoscience Laboratory, Panvel, ONGC was referred to take up microbiological studies of these cooling tower water samples in order to study the microbial load /invasion in the system and to check the efficacy of presently dosed biocide (i.e. every 10 days altenatively at 50 ppm) and to provide recommendations/suggestions for proper monitoring and better microbial control.

## EXPERIMENTAL PROCEDURE

Water samples for microbiological studies were collected in sterilized bottles as per standard procedure for investigations of bacterial studies. The samples along with makeup water were collected from 7 different sources of Uran Plant cooling towers as tabulated below:

**Table 1: Samples Source**

| Sample | Type of Sample | Sample Source |
|---|---|---|
| Sample 1 | Make up water | - |
| Sample 2 | Water Sample | LPG-I Inlet |
| Sample 3 | Water Sample | LPG-I Outlet |
| Sample 4 | Water Sample | LPG-II Inlet |
| Sample 5 | Water Sample | LPG-II Outlet |
| Sample 6 | Water Sample | C2-C3 Inlet |
| Sample 7 | Water Sample | APU Inlet |
| Sample 8 | Water Sample | APU Outlet |

Samples were inoculated for enrichment/enumeration and detection of TVC, SRB and kept in the incubator at $35^0$ C, immediately after receiving. Observations for TVC from an agar medium were recorded after 4 days of incubation and after 28 days for SRB in order to find out the bacterial load in the system. Isolation of colonies, microsopy, Gram staining were performed for bacterial identification. BioMérieux- Analytical Profile Index (API) Kit - a web based approach was used for species level identification of isolated colonies. Based on guidelines to use API kit following kits were used for identification: API CH 50B/E (*Bacillus* and related genera, *Enterobacteriaceae* and *Vibrionaceae*), API 20 NE (non-fastidious, non-enteric Gram-negative rods), API 20 E (*Enterobacteriaceae* and other non-fastidious Gram-negative rods), API Staph(staphylococci, micrococci and related genera).

Efficacy of six biocides samples provided by Uran Plant from two different manufacturers (three biocide samples for LPG-I, II and C2-C3 cooling water; other three for APU cooling water) against General Aerobic Bacteria (GAB) and Sulphate Reducing Bacteria (SRB) cultures developed from each of the sample was studied using Minimum Inhibitory Concentration (MIC) test at 50, 80,100, 150 and 200 ppm doses. Results of MIC tests were recorded after 4 days & 28 days of incubation for GAB and SRB respectively, against biocides Quat (Quaternary ammonium type), MBT (Methylene Bis Thiocyanate) and DBNPA (2,2 Dibromo-3 nitrilo propionamide).

## INVESTIGATIONS / RESULTS

Observations on different samples based on the classification indicated in the methodology and type of investigations / studies are recorded in different tables as listed below:

**Table-2: Physical observation of samples**

| Sample Source | Physical Observation |
|---|---|
| Make up water | Clear with some suspended matter |
| LPG-I Inlet | Brownish and turbid with suspended matter |
| LPG-I Outlet | Brownish and turbid with suspended matter |
| LPG-II Inlet | Brownish and turbid with suspended matter |
| LPG-II Outlet | Brownish and turbid with suspended matter |
| C2-C3 Outlet | Brownish Turbid with suspended matter |
| APU Inlet | Clear solution |
| APU Outlet | Clear solution |

**Table 3: Enumeration of Microbes**

Enumeration of microbes from samples inoculated on GAB, TVC and SRB shows following results:

| Test ⟹ Sample ⇩ | GAB Counts (No. per ml) | TVC (cfu per ml) | SRB Counts (No. per ml) |
|---|---|---|---|
| Make up water | $10^2$ | 5x $10^2$ | Nil |
| LPG-I Inlet | $10^8$ | 4.5 x $10^8$ | $10^1$ |
| LPG-I Outlet | $10^8$ | 3.8x$10^8$ | $10^1$ |
| LPG-II Inlet | $10^4$ | 54x$10^5$ | Nil |
| LPG-II Outlet | $10^4$ | 38x$10^5$ | Nil |
| C2-C3 Outlet | $10^8$ | 2.7x$10^8$ | Nil |
| APU Inlet | $10^3$ | 19X$10^3$ | $10^1$ |
| APU Outlet | $10^4$ | 6.3 X $10^4$ | $10^1$ |

**Table 4 : Microscopic Examination of isolated colonies**

7 different types of colonies are isolated from the cooling tower sample and the microscopic examination are as follows:

| Colony Type | Colony Character/ Type | Motility of isolated colonies | Gram Staining |
|---|---|---|---|
| Sample I | Orange, round shaped, sticky colonies. | Motile Rods(Bacilli) | Gram -ve |
| Sample II | Round shaped, translucent, mucoid, sticky colonies | Actively motile (short) coccobacilli | Gram -ve |
| Sample III | Rough, opaque, irregular, submerged colonies. | Motile coccobacilli | Gram -ve |
| Sample IV | Spreaded,translucent, sticky, broad, irregular colonies. | Motile Rods | Gram -ve |
| Sample V | Opaque, yellow, round shaped colonies | Non Motile cocci in bunches | Gram +ve |
| Sample VI | Colourless, translucent, round submerged colonies. | Motile coccobacilli | - |
| Sample VII | Opaque, creamy, elongated, small colonies. | Motile coccobacilli | Gram -ve |

**Table 5: Selection of API kit for species level Identification**

| Sample | Catalase Test | Oxidase Test | API kit Used | Identified Species |
|---|---|---|---|---|
| Sample I | Positive | Negative | API 50 CHB/E | *Serratia marcescens* |
| Sample II | Positive | Positive | 20E/NE | *Pseudomonas putida/ fluorescense* |
| Sample III | Positive | Positive | 20E/NE | *Pseudomonas aurogenosa* |
| Sample IV | Positive | Positive | 20NE | *Aeromonas sobria* |

| | | | | |
|---|---|---|---|---|
| Sample V | Positive | - | 20 Staph | *Staphylococcus* |
| Sample VI | - | - | - | - |
| Sample VII | Positive | Negative | 20 E | *Stenotrophomonas maltophilia* |

**Table 6: Minimum inhibitory concentration test of Biocide QUAT on GAB cultures developed from cooling waters of LPG-I Inlet , LPG-I Outlet, LPG-II Inlet, LPG-II Outlet, C2-C3, APU Inlet APU outlet and Make up water**

| **Biocide QUAT** | 50 ppm | 80 ppm | 100ppm | 150 ppm | 200ppm |
|---|---|---|---|---|---|
| Make up water | ++ | ++ | ++ | -- | -- |
| LPG-I Inlet | ++ | ++ | ++ | ++ | ++ |
| LPG-I Outlet | ++ | ++ | ++ | ++ | -- |
| LPG-II Inlet | -- | -- | -- | -- | -- |
| LPG-II Outlet | -- | -- | -- | -- | -- |
| C2-C3 Outlet | ++ | ++ | ++ | -- | -- |
| APU Inlet | ++ | ++ | ++ | -- | -- |
| APU Outlet | ++ | ++ | ++ | -- | -- |

**Table 7: Minimum inhibitory concentration test of Biocide MBT and biocide DBNPA on GAB cultures developed from cooling waters of LPG-I Inlet , LPG-I Outlet, LPG-II Inlet, LPG-II Outlet, C2-C3, APU Inlet APU outlet and Make up water**

| | **Biocide MBT** | | | | **Biocide DBNPA** | | | |
|---|---|---|---|---|---|---|---|---|
| **Doses** | 50 ppm | 100 ppm | 150 ppm | 200 ppm | 50 ppm | 100 ppm | 150 ppm | 200 ppm |
| Make up water | -- | -- | -- | -- | -- | -- | -- | -- |
| LPG-I Inlet | -- | -- | -- | -- | ++ | -- | -- | -- |
| LPG-I Outlet | -- | -- | -- | -- | -- | -- | -- | -- |
| LPG-II Inlet | -- | -- | -- | -- | -- | -- | -- | -- |
| LPG-II Outlet | ++ | ++ | -- | -- | ++ | -- | -- | -- |
| C2-C3 Outlet | -- | -- | -- | -- | -- | -- | -- | -- |
| APU Inlet | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| APU Outlet | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |

**Table 8: Minimum inhibitory concentration test of Biocide Quat, MBT and DBNPA on SRB cultures developed from cooling waters of LPG-I Inlet, LPG-I Outlet, APU Inlet and APU Outlet**

| | **Biocide QUAT** | | | **Biocide MBT** | | | **Biocide DBNPA** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Doses** | 80 ppm | 100 ppm | 150 ppm | 80 ppm | 100 ppm | 150 ppm | 80 ppm | 100 ppm | 150 ppm |
| LPG-I Inlet | -- | -- | -- | ++ | -- | -- | -- | -- | -- |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| LPG-I Outlet | ++ | ++ | -- | -- | -- | -- | ++ | -- | -- |
| APU Inlet | ++ | -- | -- | ++ | ++ | ++ | ++ | ++ | -- |
| APU Outlet | ++ | -- | -- | ++ | ++ | -- | ++ | ++ | -- |

## OBSERVATIONS AND DISSCUSSION

- TVC in make up water, LPG-I, LPG-II, C2-C3 and APU samples are in the range of 5 x $10^2$ per ml to 4.5 x$10^8$ cfu per ml. SRB detected in LPG-I and APU are 10 per ml, while LPG-II, make up water and C2-C3 samples did not show SRB counts (Table-3)
- Total 7 different types of colonies were observed in different cooling tower samples. These colonies were isolated and microscopic examinations were carried out and results are tabulated in Table-4.
- Species level identification of isolated colonies using API kit showed presence of Serratia marcescens, Pseudomonas putida/ fluorescense, Pseudomonas aurogenosa, Aeromonas sobria, Staphylococcus, Stenotrophomonas maltophilia species.(Table-5).
- Results of MIC of biocides with GAB and SRB cultrures are shown in table 6 to 8.
- Biocide Quat is effective at 50 ppm against GAB cultures from LPG-II and at 150 ppm for GAB from Make up water, C2-C3 and APU.For LPG-I Outlet GAB culture it is not effective up to 200 ppm dose.(Table 6)
- Biocide MBT is effective for GAB cultures developed from make up water, LPG-I Inlet & Outlet, LPG-II Inlet and C2-C3 at 50 ppm , at 150 ppm in case of LPG-II Outlet, while for APU Inlet & Outlet it is not effective upto 200 ppm. (Table 7)
- Biocide DBNPA is effective at 50 ppm for GAB cultures developed from make up water, LPG-I outlet, LPG-II Inlet, C2-C3 and at 100 ppm for LPG-I Inlet and LPG-II Outlet, while for APU Inlet & Outlet it is not effective upto 200 ppm. (Table 7)
- Effective dose of biocide QUAT against SRB, for LPG-I Inlet is 80 ppm, for LPG –I Outlet it is 150 ppm and for APU Inlet & Outlet it is 100 ppm.( Table 8)
- Biocide MBT is effective at 100 ppm, against SRB culturs developed from LPG-I Inlet, at 80 ppm for LPG-I Outlet and effective at 150 ppm for APU Outlet while it is not effective upto 150 ppm for SRB developed from APU Inlet sample. (Table 8)
- Biocide DBNP is effective for SRB cultures developed from LPG-I Inlet at 80 ppm, LPG-I Outlet at 100 ppm and for APU Inlet & Outlet at 150 ppm. (Table 8)

## CONCLUSION

- The cooling water samples of LPG-I, LPG-II and C2-C3 contained high numbers and consequent biofouling that may lead to biocorrosion due to heavy bacterial load in the system.
- As per scheduled dosing plan of Uran Plant, these biocides are used for treatment every 10 days altenatively at 50 ppm dose. But cultures developed from various samples showed variation in their sensitivity towads the three biocides hence required different doses to control. Biocides being used in APU cooling tower were not efficient in controlling microbial growth upto 150ppm.
- Therefore, regular monitoring and biocide treatment with optimum dose of biocides is essential for trouble free performance.
- Treatment with Biocide MBT, DBNPA and Quat at recommended doses may keep the bacterial counts within permissible limits.

## RECOMMENDATION/SUGGESTION

- Certain standard practices are followed internationally, for the proper functioning of cooling towers, with respect to cleaning and disinfection:
  Cooling towers may be physically cleaned every three months; unless another cleaning frequency is approved. Cooling towers should be kept free of any extraneous matter of, plant animal or inorganic origin that may adversely affect the equipment or increase the risk to public health. Cooling towers may be cleaned and disinfected before commissioning & before each start up after extended shutdown periods.

  Acceptable results indicating good microbial control would be less than or equal to $10^4$cfu/ml. Results showing levels between$10^4$-$10^5$ cfu/ml (colony forming units) indicate that system may be going out of control & should serve as a cautionary warning. Levels > $10^5$cfu/ml indicate a cooling system is out of microbial control.

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